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Repellent and Oviposition-Deterring Effects of Hop Beta-Acids on the Two-Spotted Spider Mite *Tetranychus urticae*

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Abstract: Colupulone, the main component of the beta-acid fraction of hop extracts, was shown to repel the two-spotted spider mite *Tetranychus urticae* Koch from the surface of plant leaves. The total beta-acid fraction was found more effective in repelling the mites and reducing the number of eggs laid than colupulone at equivalent concentrations, suggesting that other components of the extract have repellent and oviposition-deterring effects.

Key words: *Tetranychus urticae*, hop beta-acids, colupulone, repellent, oviposition deterrent.

1 INTRODUCTION

Naturally occurring 2-acylcyclohexane-1,3-diones or beta-triketones are uncommon but have been found in a few plant species[1,2] including hops (*Humulus lupulus* L. Cannabinaceae), and more recently in two species of insects.[3,4] These compounds are fully enolised, weakly acidic and generally have some form of antibiotic activity. Synthetic 2-acylcyclohexane-1,3-diones have been prepared that are antibiotic,[5] fungicidal[6] or acaricidal.[7] Both the alpha- and beta-acids from hop are 2-acylcyclohexane-1,3-diones, so the beta-acid fraction of hop extracts, which is a by-product of hop processing for brewing, was examined for its effect on the feeding behaviour of the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). Earlier studies[8] had shown that hop beta-acid fraction was repellent and affected both the feeding and oviposition behaviour of the mite. The aim of the present study was to determine whether this activity could be accounted for by colupulone (Fig. 1), the chief beta-acid component of the extract, as part of an attempt to develop natural products, acting as antifeedants, for use in crop protection.[9]

2 MATERIALS AND METHODS

A strain of *T. urticae* resistant to several commercial acaricides and originating from a glasshouse at East Malling, was reared on French beans (*Phaseolus vulgaris* L., cv. Canadian Wonder) at 20°C. Ethanolic solutions of the beta-acid fraction (Scottish & Newcastle Breweries) and colupulone (HRI Wye) were used for the bioassays.

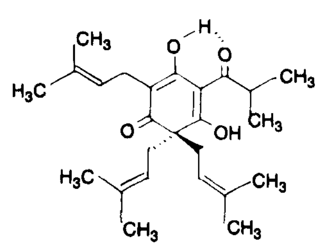

Fig. 1. Colupulone.

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2.1 High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS) analyses

All HPLC analyses were done on a Spherisorb 5 ODS2 column (25 cm × 4·6 mm) using methanol + water (80 + 20 by volume) containing either orthophosphoric acid (1·0 g litre^{-1}) or trifluoroacetic acid (1·0 g litre^{-1}) at 1 ml min^{-1}. Samples were injected using a Rheodyne 7125 injector fitted with a 20-μl loop and detected with a Holochrome UV detector operated at 314 nm. The beta-acid fraction, a sticky gum, was dissolved in methanol and its colupulone content determined from the area of its HPLC peak obtained under the above conditions by reference to a calibration curve constructed using colupulone (colupulone 98%, lupulone 2%, by HPLC and GC-MS analysis) under the same HPLC conditions. The colupulone content was 6% by weight of the crude beta-acid fraction.

Colupulone isolated from the beta-acid fraction by HPLC was identified by GC-MS using a Hewlett-Packard 5890 gas chromatograph coupled directly to a VG 70-250 double-focusing mass spectrometer (70 eV, source temp. 250°C). Separations were achieved by on-column injection on a J&W fused silica capillary column (30 m × 0·32 mm) coated with DB1 film (0·25 μm) operated isothermally at 30°C for 5 min and programmed at 5°C min^{-1} to 180°C, then 1°C min^{-1} to 225°C and then at 5°C min^{-1} to 300°C. The retention time of colupulone was 48 min with a carrier gas flow of 2 ml min^{-1} helium into the mass spectrometer.

2.2 Bioassays

Repellent and oviposition-deterrent effects of the beta-acid fraction and colupulone was determined in a choice bioassay by applying ethanolic solutions to French bean leaf discs 5 cm in diameter. These were maintained on moist cotton wool at 20°C, and 16 : 8 h light : dark photoperiod.

All solutions were applied with an APE80 electrostatic sprayer[10] using the following conditions; velocity 0·4 m s^{-1}, flow rate 24 ml min^{-1}, charge 30 kV, height 25 cm, swathe width 1·0 m and application rate equivalent to 10 litre ha^{-1}. Each leaf disc was divided into two halves by an ink line drawn across its surface. Tissue paper with a straight edge was placed along the line, and spray was applied to the uncovered half of the disc and allowed to dry. Clean tissue was then placed up to the line on the sprayed half of the disc and spray applied to the remaining side. A total of seven treatments were applied to the leaf discs, 100, 10 and 1·0 g litre^{-1} beta-acid fraction in ethanol *v* ethanol, 6, 0·6 and 0·06 g litre^{-1} colupulone in ethanol *v* ethanol and ethanol *v* no treatment. In the ethanol *v* no treatment only one half of the disc was sprayed. After spraying, a narrow band of polymeric gum ('Tanglefoot'; Tanglefoot Co., Grand Rapids MI, USA) was placed round the circumference of each disc.

Ten adult females were placed on each disc and the number of mites surviving and number of eggs laid after 18, 40, 64 and 88 h were counted. Each treatment was replicated ten times.

2.3 Statistical analysis

To assess the repellent effect of the treatments, the percentage of live mites that were on the treated side of the leaf disc at each inspection was calculated. This percentage was compared between treatments, and was also compared directly with the nominal value, 50%, that would be expected in the absence of any repellent effect. A binomial analysis of deviance was used, with allowance for overdispersion where necessary.[11] Strictly, such an analysis leads to a separate standard error of difference (SED) for every pair of treatments, but following common practice, a single pooled SED is presented in the tables that follow. Binomial analysis of deviance was also used to analyse mite survival and to investigate possible oviposition deterrent effects of the treatments.

3 RESULTS

3.1 Repellent effect

Mites settled with equal readiness on both halves of the leaf discs in the ethanol *v* no treatment assay (Table 1).

At the first inspection (18 h) the percentage of live mites on the treated side of the leaf disc was significantly lower for the beta-acid fraction treatments than for the corresponding colupulone treatments ($P < 0{\cdot}05$), except at the lowest concentration, when neither differed significantly from 50%. At subsequent inspections there was no significant difference between beta-acid and colupulone.

At the first (18 h) and second (40 h) inspections there were significant effects of concentration ($P < 0{\cdot}01$), with the percentage of mites on the treated side of the leaf disc decreasing with increasing concentration for both the beta-acid fraction and colupulone treatments (Fig. 2). At the third (64 h) and fourth (88 h) inspections there was no longer any significant effect of concentration, although the lowest percentages were again observed at the highest concentration and some of these differences remained significantly less than 50% (Table 1).

3.2 Proportion of mites still alive

After 18 h, 86% of the mites were still alive on discs treated with beta-acid fraction or colupulone. By the end of the experiment this had decreased to 54%, compared to 71% on the ethanol *v* no treatment controls.

TABLE 1
Percentage of Live Mites on the Treated Side of the Leaf Discs[a]

Treatment (g litre^{-1})	*18 h*	*40 h*	*64 h*	*88 h*
Beta-acid fraction				
100	7***	21***	32*	32
	(5/74)	(14/67)	(17/53)	(11/34)
10	21**	35	36	47
	(14/67)	(20/57)	(15/42)	(16/34)
1·0	60	60	52	45
	(55/91)	(49/81)	(37/71)	(29/65)
Colupulone				
6·0	26**	36*	35	31*
	(22/84)	(26/73)	(22/63)	(17/54)
0·6	43	40	49	40
	(38/88)	(30/75)	(30/61)	(23/58)
0·06	60	55	46	49
	(56/93)	(47/85)	(35/76)	(34/69)
Ethanol *v* untreated				
	42	44	45	48
	(38/90)	(36/81)	(33/73)	(34/71)
SED (59 d.f.)	9·1	9·2	11·0	11·9

[a] Asterisks indicate that the percentage was significantly different from 50%: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

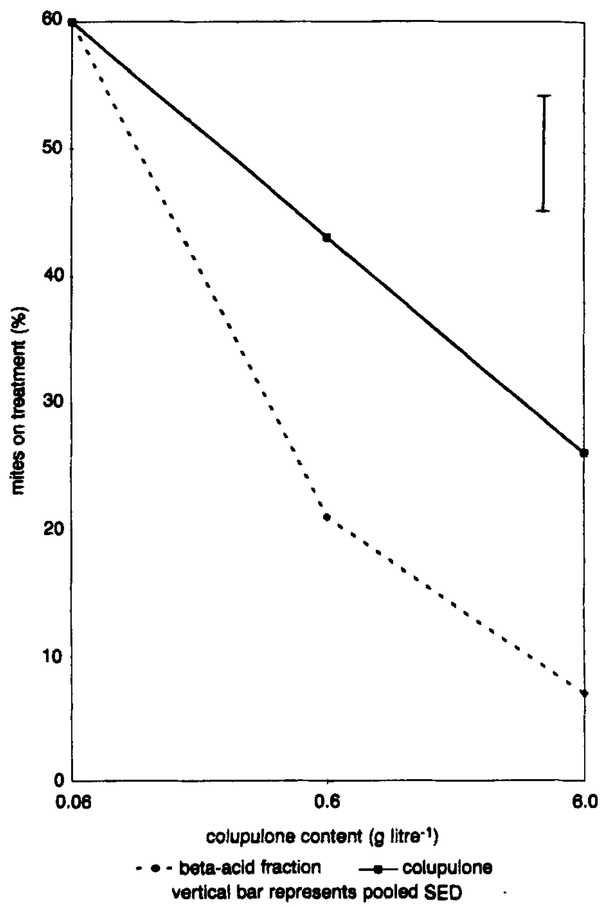

Fig. 2. Percentage of total mites on beta-acid fraction and colupulone treatments after 18 hours.

This difference resulted primarily from reduced survival at the two higher concentrations of the beta-acid fraction (Table 2).

3.3 Proportion of dead mites that died off the leaf

The proportion of dead mites that died off the leaf was calculated as an additional measure of repellency. The treatment was applied to only one half of the leaf disc so that mites repelled by the treatment could move to the control half. If they were greatly repelled, they could 'run off' into the 'Tanglefoot' ring in an attempt to escape.

Overall, the numbers of mites that ran off did not differ significantly between the beta-acid fraction and colupulone treatments. There was also no effect of concentration. The highest incidence of run-off was at the start of the experiment. After 18 h, 84% of dead mites were found in the Tanglefoot. However, only 82 mites had died by this time (from a total of 700). Mites continued to run off throughout the experiment, and after 88 h, run-off had accounted for 54% of total deaths (Table 3).

3.4 Oviposition deterrent effect

Throughout the experiment, the proportions of eggs on the disc halves treated with 100 and 10 g litre^{-1} beta-acid fraction were significantly lower than on their controls. Slightly more eggs were found on both the 1·0 g litre^{-1} beta-acid fraction and 0·06 g litre^{-1} colupulone treatments than on their controls (Fig. 3), but these differences were generally not significant (Table 4).

During the first time period (18 h), the proportion of eggs on the beta-acid faction and colupulone treated leaf halves decreased with concentration ($P < 0{\cdot}001$),

TABLE 2
Percentage of Mites Alive after 88 Hours (from a Total of 100)

Treatment	*Low*	*Medium*	*High*
Beta-acid fraction	65	38	34
Colupulone	69	58	60
Control	71		
SED (52 d.f.)	9·2		

TABLE 3
Percentage of Dead Mites in the Tanglefoot Ring

Time (h)	*18*	*40*	*64*	*88*
	84	67	61	54
	(69/82)	(95/142)	(129/213)	(144/266)

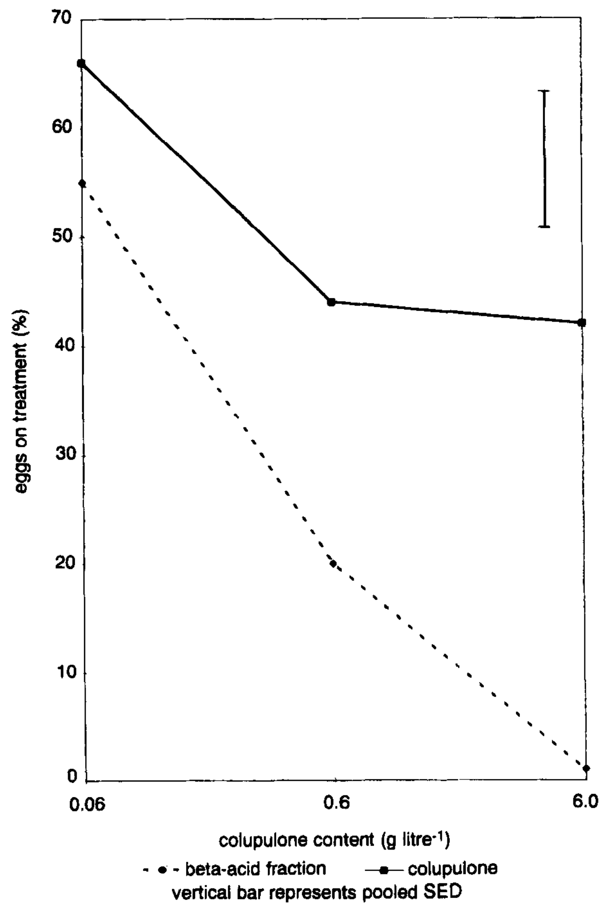

Fig. 3. Percentage of total eggs on beta-acid fraction and colupulone treatments after 18 hours.

most obviously on the 100 g litre^{-1} beta fraction treatment (Fig. 3), with just two eggs on the treated disc halves compared with 179 on the adjacent ethanol-sprayed halves.

4 DISCUSSION

The results showed that both the highest concentrations of culupulone and beta-acid fraction repelled spider mites, and also affected their survival. The effect was most marked after time period one (18 h), when the beta-acid fraction was also significantly more repellent than colupulone. However, the difference was not significant after time period two (40 h). This suggests that coulupulone is not the only active component in the beta-acid fraction and that another volatile or rapidly oxidised component may have been responsible for the initial difference between the treatments.

The most dilute concentrations of beta-acid fraction and colupulone were not repelled to mites, indeed they may have been mildly attractive, although not significantly so. Similarly, egg numbers were not reduced on these treatments.

The greatest difference between the responses to colupulone and beta-acid treatments was seen in the oviposition of the mites. Significantly fewer eggs were found on the 100 and 10 g litre^{-1} beta-acid fraction treatments throughout the experiment, whereas none of the colupulone treatments produced a statistically signifi-

TABLE 4
Percentage of Eggs on Treated Half of Discs[a]

Treatment (*g litre*$^{-1}$)	*18 h*	*40 h*	*64 h*	*88 h*
Beta-acid fraction				
100	1***	13***	24***	25***
	(2/81)	(40/315)	(92/388)	(105/423)
10	20*	21**	29**	31**
	(26/126)	(49/235)	(96/330)	(110/360)
1·0	55	56	56	56
	(110/200)	(250/447)	(360/638)	(451/811)
Colupulone				
6·0	42	41	44	47
	(61/145)	(138/338)	(205/466)	(278/586)
0·6	44	44	47	45
	(87/198)	(158/363)	(240/512)	(309/682)
0·06	66	65*	57	54
	(127/192)	(229/354)	(308/541)	(376/700)
Ethanol *v* untreated				
	62	58	44	46
	(136/216)	(170/292)	(305/701)	(421/910)
SE	12·4	9·3	7·6	7·3
(59 d.f.)				

[a] Asterisks indicate that the percentage was significantly different from 50%: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

cant decrease in oviposition, despite some of them repelling mites. At all times the differences in oviposition on the beta-acid fraction and colupulone treatments were significant.

The treatment effects were proportional to concentration, as shown in Figs 2 & 3. Extrapolation of the lines suggests that it may not be possible to repel the mites totally, but a higher concentration of beta fraction could prevent oviposition. However, solutions containing more than approximately 25% tend to result in sticky deposits on the leaf surface, which form a physical barrier to the mites.

The difference in activity between the colupulone and beta-acid fraction treatments must be due to additional components in the beta-acid fraction, since both treatments contained similar levels of colupulone. All hop acids are readily oxidised at room temperature to a wide variety of oxidation products.[12] The beta-acid fraction used in these studies contained colupulone, numerous complex oxidation products derived from the beta-acids, plus small amounts of a larger number of other components (Mudd, A. & Maniar, S. P., unpublished). Further separation of the beta-acid fraction and subsequent bioassay of the products is required to account for the difference in the repellent and oviposition-deterrent activity between colupulone and the beta-acid fraction.

5 CONCLUSIONS

The higher concentrations of the beta-acid fraction and colupulone repelled mites and reduced their survival. Oviposition was significantly reduced by the beta-acid fraction but not by the colupulone treatment. Colupulone is an active component of the beta-acid fraction but not the only one. Further work is required to identify the other active components of the beta-acid fraction.

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